Ngn3 is both necessary and sufficient to induce endocrine islet cell differentiation from endodermal progenitor cells during embryogenesis. Because robust *Ngn3* expression has not been detected in hormone-expressing pancreatic islet cells, Ngn3 is utilized as an endocrine progenitor marker and regarded dispensable for the function of differentiated islet cells. Thus, detection of *Ngn3* expression in the adult pancreatic cells was interpreted as evidence of the presence of endocrine progenitors or stem cells. Here we utilized *Ngn3-CreER* knock-in reporter mice and mRNA/protein-based assays to examine *Ngn3* expression in hormone-expressing islet cells. We showed that Ngn3 mRNA and protein are detected in hormone-producing cells at both embryonic and adult stages. Significantly, inactivating Ngn3 in insulin-expressing β cells at embryonic stages or in Pdx1-expressing islet cells in the adults impairs endocrine function, a phenotype that is accompanied by a reduced expression of several Ngn3 target genes that are essential for islet cell differentiation, maturation, and function. These findings demonstrate that Ngn3 is required for not only initiating endocrine cell differentiation, but also islet cell maturation and functional maintenance, and Ngn3 production in the adult pancreatic cells cannot be utilized as an endocrine progenitor marker.

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Program/Abstract # 406
Erythroid development in the absence of hemoglobin
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The mammalian erythrocyte is a highly specialized blood cell that differentiates via an orderly series of committed progenitors in the bone marrow in a process termed as erythropoiesis. In mature red blood cells over 95% of the protein is hemoglobin (Hb) that consists of two alpha and two beta globin polypeptide chains. What happens during erythropoiesis in the absence of hemoglobin? To answer this question, we generated homozygous alpha and beta globin knockout (Null Hb) embryos, adult chimeric mice using novel Null Hb embryonic stem cells (Null Hb ES), and an in vitro ES cell derived erythroid progenitor (ES-EP) culture system. Null Hb embryos died at ~12.5 d in utero. Committed Null Hb erythroid progenitors were present, but did not differentiate beyond the basophilic erythroblast stage. EKLF was tagged by EGFP to track Null Hb ES cells derived from erythroid cells in chimeras. Analysis of adult chimeric bone marrow revealed that Null Hb derived white blood cells developed normally, but the erythroid lineage was again blocked at the basophilic erythroblast stage. In vitro Null Hb ES-EP cultures could support the growth and expansion of Null Hb proerythroblasts; however, upon terminal differentiation Null Hb ES-EP cells undergo apoptosis and cell death. Expression of human myoglobin targeted to the beta globin locus in Null Hb ES cells could rescue erythroid development in the bone marrow of chimeras. These experiments demonstrate that Hb is not necessary for erythroid lineage commitment, is required for terminal erythroid differentiation, and that human myoglobin can rescue erythroid development in the absence of Hb.

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Program/Abstract # 407
Basal cells as stem cells of the mouse trachea and human conducting airways
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The epithelial cells of the respiratory epithelium of mice and humans, constantly exposed to inhaled toxins and pathogens, are maintained over the long term via controlled division of adult tissue stem cells. We have demonstrated that basal cells (BCs) of the mouse trachea give rise to both Clara and ciliated cells by in vivo lineage tracing. Furthermore, we have developed a novel culture system to assay the self-renewal and differentiation of BCs. To identify mechanisms that regulate these behaviors, we have purified BCs by fluorescence activated cell sorting and performed microarray analysis. Using mutant mice and in vitro assays, we are currently testing the hypothesis that genes expressed at high levels in BCs, including transcription factors, signaling molecules, and cytoskeletal components, control their proliferation, differentiation, and motility both at a steady-state and in response to epithelial injury. Finally, we have determined that p63-expressing cells are present even within the smallest conducting airways of humans. Characterization of this stem cell population in mice and humans should enhance our understanding of pathological conditions of the airways including chronic asthma, chronic obstructive pulmonary disease, and cancer.

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Program/Abstract # 408
Bone marrow-derived macrophages fuse with intestinal epithelium in the stem cell niche after injury
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Adult bone marrow-derived cells (BMDCs) can engraft into damaged intestinal epithelium of mice representing a potential avenue for facilitating tissue regeneration. The underlying mechanism for this BMDC engraftment occurs by cell fusion, analogous to cell fusion that occurs during development. We previously identified the intestinal stem cell as the fusion target, but the marrow-derived fusion partner remains unknown. Here we identified the macrophage population as the primary BMDC fusion partner by isolation and transplantation of discrete hematopoietic lineages into recipient mice. Transplantation of isolated macrophages supported robust intestinal epithelial fusion at levels equivalent to whole bone marrow. Additionally, a close examination of the time course for cell fusion reveals that macrophages are among the first cell types recruited to the intestine after injury and surround the stem cell niche. Interestingly, the fusion hybrid cells are not multinucleate, indicating they may be reprogrammed. Indeed expression of macrophage genes was sustained in long-lived fusion hybrids. These studies are the first to illustrate the critical temporal window for visualization of cell fusion after injury. Importantly, understanding the timing and cellular players involved in cell fusion establishes the foundation for further investigations into the molecular mechanism. Establishing the temporal dynamics of fusion and subsequent genetic reprogramming in cell fusion hybrids may provide insight into the physiologic impact of cell fusion in regeneration and susceptibility to disease.

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Program/Abstract # 409
Identifying gene regulatory networks that control adult regeneration in zebrafish
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Zebrafish can regenerate an impressive array of organs including the heart, spinal cord and fins. While much work has been done to elucidate the signaling molecules that initiate regeneration, little is known about the transcriptional changes that are necessary for repair to occur. In order to understand the molecular mechanisms driving the regeneration of complex tissues, we are attempting to identify transcriptional gene networks that regulate caudal fin regeneration in the adult zebrafish. We have carried out a candidate-based quantitative RT-PCR screen to discover transcriptional regulators which are expressed after caudal fin injury. A detailed time course of expression analysis revealed several novel genes induced during regeneration. We have confirmed these results using in situ hybridization, and determined the expression domains of these genes within the regenerating fin. Currently, we are creating a variety of tools in order to understand the function of these transcriptional regulators. We have made transgenic lines that express GFP-tagged versions of the genes expressed either from their native promoters or with the hsp70 heat shock overexpression system. In addition, we are using morpholinos to knock down the expression of these genes at several time points during regeneration. We are also optimizing chromatin immunoprecipitation and high thoughput sequencing (ChIP-Seq) from adult regenerating tissues so that we may ultimately place these transcription factors into regulatory networks.

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Program/Abstract # 410
Comparative analysis of satellite cells from blastema and adult tissues of the electric fish *S. macrurus*

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The vertebrate *S. macrurus* can regenerate muscle and electric organ (EO) after repetitive tail amputations. Our ultrastructural and immunolabeling studies demonstrated that satellite cells (SCs) in muscle and EO contribute to blastema formation and regeneration of myogenic tissues (Weber, unpub). To understand some of the cellular mechanisms responsible for its robust regeneration, we have begun to compare SCs derived from muscle, EO and regeneration blastema tissues regarding their proliferation and differentiation potential. We modified a protocol for isolating satellite cells in rainbow trout by Fauconneau et al. (2000) to establish primary SC cultures from muscle, EO and regeneration blastema (Archer et al., 2008). BrdU incorporation studies using cells in growth medium (9 d) showed no obvious differences in the proliferation capacity of SCs from the different tissues. A fraction of all SCs in differentiation medium (5 d) were multinucleated. Some mono- and multinucleated cells were immunolabeled with antibodies against muscle markers. Blastema SCs seemed to form a greater number of myotubes than SCs isolated from either muscle or EO. Myotubes formed from blastema SCs were also generally longer and thinner than those derived from muscle or EO SCs. Further in vivo and in vitro analyses will determine whether the current findings reflect biological differences between distinct populations of SCs in their competence to differentiate into myofibers and contribute to adult tissue regeneration. Establishment of a myogenic SC line from *S. macrurus* will facilitate our developmental studies on the origin of the EO.

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Program/Abstract # 411
Intestinal renewal and regeneration in the planarian *Schmidtea mediterranea*

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In planarians, pluripotent somatic stem cells called neoblasts continuously replenish tissues during normal cellular turnover and regenerate organ systems after injury, but these processes are not well understood at the cellular or molecular level. We have characterized the dynamics of cell renewal and regeneration in the intestine of the planarian *Schmidtea mediterranea*, utilizing a variety of immunohistochemical methods and live animal imaging in detailed time course analyses. As expected, intestinal epithelial cells do not actively cycle, but rather neoblasts differentiate into intestinal cells both in uninjured animals and during regeneration. Additionally, differentiated intestinal tissue undergoes significant remodeling/repatterning in amputated fragments as polarity and symmetry of the intestinal branches are restored. We have developed a novel method for purification of intestinal phagocytes, allowing the generation of a panel of monoclonal antibodies as well as microarray-based identification of over 1000 genes that are differentially expressed in the intestine. An RNAi screen is underway, focused on candidates that may regulate intestinal renewal, remodeling/regeneration, metabolism, and neoblast proliferation or differentiation. (This work was supported by NIH R01 HD043403 to PAN, and NIH-NIDDK F32 DK077469 to DJF.)

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Program/Abstract # 413
Dynamic expression of planarian Wnt genes reveals complex response to amputation

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Wnt signaling via β-catenin is known to play crucial roles throughout animal life, from early development to adulthood. In planarians, animals with astounding regenerative abilities, β-catenin functions to maintain and specify AP identity in adults and regenerating animals. While Wnt ligands are expressed in a complex posterior to anterior gradient in intact animals, expression during early phases of regeneration remains unknown. We report that Wnt genes are expressed in dynamic, location-specific bursts after amputation. We show that these transcriptional responses are mounted by pre-existing tissues, and are separate from the expression patterns observed as stem cells proliferate to replace missing structures. As the internal anatomy remodels to establish proper size and proportion, we find an interdependence between new and old tissue to establish proper spatial control of Wnt expression. Thus, cells distributed throughout the planarian body plan appear to evaluate and respond to their new position in an amputated fragment. We suggest that the Wnt expression patterns described in intact planarians represent only a small slice of the complex and dynamic signaling that occurs early during a regenerative event.

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